structural difference to separate generically all Judith River, Belly River and Two Medicine trachodonts from those obtained in the Lance formation, and that therefore the use of the term *Trachodon* should be restricted in its application to some one of those trachodonts found in the older beds.

3. That the restriction of the genus *Claosaurus* to the Niobrara species *C. agilis* Marsh first proposed by Hatcher be endorsed.

4. That *Claosaurus annectens* Marsh should be regarded as a synonym of *Thespesius occidentalis* Leidy as first proposed by Lucas.

CHARLES W. GILMORE
U. S. NATIONAL MUSEUM,
January, 1915

THE SOCIETY OF AMERICAN BACTERIOLOGISTS. II

Technique

Under the supervision of G. F. Ruediger

The Bacteriological Work of the Bureau of Chemistry and Its Possibilities: CHARLES THOM.

The papers presented by members of the bacteriological staff of the Bureau of Chemistry are fairly representative of the manner in which numerous problems arising from the enforcement of the Food and Drugs Act are being met by the bacteriological laboratory. Very many of the food products and other preparations met with in inspection work have not been adequately studied by bacteriologists. No analysis of the flora present in such substances is available. Standard methods for testing them have not been developed. The workers into whose hands they fall must then make a full study of several to many brands of the commercial article and very frequently follow the product every step of the way back to the actual producer before adequate data can be obtained to determine what action, if any, shall be taken by the bureau. The members of the Bacteriological Society are earnestly requested to aid this work whenever opportunity arises by studying the bacteriological conditions obtaining in food-stuffs and the standardization and publication of methods of procedure.

In addition to its inspection work, the bureau is now establishing a research laboratory to take up food deterioration, fermentation and technically bacteriological and mycological work upon unsolved problems concerning foods and drugs. This work will be carried in the closest possible cooperation with the chemical laboratories of the bureau dealing with the same related problems. By these two methods of attack it is hoped to enlarge our knowledge of the flora of food stuffs and the relation of these organisms to normal and abnormal conditions as found.

Methods of Counting Bacteria: ROBERT S. BREED.

Three methods of counting the number of bacteria present in various substances have been generally recognized. In order of their historical development, they are the microscopical method, the dilution method and the plating method. For the past few years, however, the latter method has been used, especially among American bacteriologists, almost to the exclusion of the others and this, in spite of the fact that what little comparative work has been done indicates that certain uncontrollable elements in this technique cause large errors.

Among other causes of irregularities in the counts, there are two which tend to lower the count in both the dilution and the plating method. One of these is the fact that the organisms present in the substance under examination may fail to grow in the culture medium used, and the other, that the clumping of the organisms reduces the number of centers from which growth occurs. The microscopical technique is free from these objections, but it is open to another in all cases where a count of living organisms only is desired. This objection arises because of the fact that it is ordinarily impossible to distinguish organisms which were alive at the time the preparation was made from those which were dead. This difficulty causes the count obtained in this way to be higher than it should be.

These conditions which have thus far proved to be uncontrollable in all of the three methods are largely responsible for the big discrepancies in the comparative counts which have been made. These discrepancies show most strikingly that all so-called bacterial counts are much better styled "estimates" than "counts." Statements that certain substances, such as milk, water, sewage and the like, contain such and such numbers of bacteria are particularly unfortunate, for they are plain misstatements of facts. In most cases the figures given represent counts of colonies on agar or gelatin and may be properly so recorded but these figures are usually far below the actual number of bacteria present.

So far as raw milk is concerned, microscopical methods of counting have been shown to have great usefulness, for, in these cases, the number of

dead bacteria present is at a minimum. Moreover, in those cases where they are present, they are just as indicative of the past history of the milk as are living bacteria. Very variable conditions in regard to the clumping of bacteria in milk have been observed. In many cases the bacteria occur largely as single individuals or as clumps of twos, in other cases the milk is filled with compact clumps which could not be separated by any known methods of plating. Where thick cream is present and the right types of bacteria occur, colonies may be formed much like those found on agar or gelatin. These variations in clumping produce very variable effects on the plate count which would be unrecognizable where this technique is used alone.

The Standard Method of Determining Nitrate Reduction: ROBERT S. BREED.

Attention is drawn to the fact, more or less generally known, that the Committee on Standard Methods of Bacterial Water Analysis have inadvertently given us two different formulæ for nitrate broth in each of the two editions of the Standard Methods which have been published. All of the formulæ call for one gram of peptone per liter, but the amount of nitrate varies. Altogether three different amounts are mentioned, namely, 2 grams, 0.2 gram and 0.02 gram per liter.

The committee's statement that the nitrate reduction test is sometimes quite erratic has been explained for some cases at least by tests which have been carried out at the New York State Experiment Station. Fifty cultures of bacilli of the colon group, isolated from a sewage-polluted stream, gave very erratic results with the standard broth which contained 0.2 gram of nitrate per liter, scarcely one third of the cultures giving results which showed a clear reduction. However, as soon as the amount of peptone was increased to five grams per liter, all of the cultures gave positive reactions for all tests.

On the other hand, tests for thirty cultures of bacilli of the *subtilis* group isolated from soil gave unmistakably positive or unmistakably negative results in a number of tests in the same nitrate broth. Varying the amount of peptone from one to five grams per liter had no influence on the results. Twenty of these cultures reduced nitrates, while ten failed to do so. In all cases there was a vigorous growth of the bacilli in every tube.

Tests with a single culture of an unknown soil organism showed, however, that it was necessary to be cautious in recommending that the amount of peptone in the standard broth be increased. This organism showed a condition the exact reverse of that just reported for the colon bacillus. Positive results were obtained in all cases where 1 gram of peptone per liter was used, while increasing the amount of peptone caused erratic and finally negative results when as much as 5 grams per liter was used.

Evidently nitrate reduction should be tested in a broth in which the organism to be tested will grow vigorously. Irregular results are open to suspicion in all cases. No one broth can be used for all organisms and suitable broths must be devised to fit each group of organisms. It is particularly unfortunate to report an organism as lacking the power to reduce nitrates when it fails to reduce them in a broth in which it does not grow. Either such results should not be reported at all or reported as doubtful.

Starch Agar, a New Culture Medium for the Gonococcus: EDWARD B. VEDDER.

Starch agar is a beef-infusion agar (1.5 per cent.) without salt or peptone, to which is added 1.0 per cent. of starch, preferably corn-starch, though potato starch or tapioca will serve. Reaction, 0.2–0.5 acid to phenolphthalein. The advantages of this medium are as follows:

1. The gonococcus grows very freely on this medium, producing a heavy growth suitable for the preparation of vaccines or antigens.

2. When the tubes are sealed with paraffine, cultures remain alive upon this medium for a long time, at least 20 days, so that transfers of stock cultures may be safely made every two weeks instead of every three or four days, as is customary when other media are used.

3. This medium may be melted and used in pour plates in order to isolate gonococci in pure culture from gonorrheal pus.

4. Some other organisms that are usually cultivated with considerable difficulty grow well on this medium; *i. e.*, certain strains of tubercle bacilli, the lepra bacillus (Duval), and freshly isolated streptococci and pneumococci.

5. The medium is suitable for routine use as practically all organisms grow as well or better on this medium as on plain agar.

The great simplicity of preparation of this medium and its many advantages appear to indicate that it may be very useful to many workers.

A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly from the Sputum and Feces, with the Aid of Sodium Hydrate and Gentian Violet-egg-meat Juice Media: S. A. PETROFF.

The object of this investigation was to devise a simple, practical and reliable method for the isolation and cultivation of the tubercle bacillus from the sputum and feces. Most of the methods employed in the last twenty years do not give uniformly positive results.

Taking into consideration the inhibitory action of gentian violet on many organisms, it was selected as the most favorable stain.

Preparation of the Media

I. *Meat Juice.*—Five hundred grams of beef or veal are infused in 500 c.c. of a 15-per-cent. solution of glycerine in water. Twenty-four hours later the meat is squeezed in a sterile meat press and collected in a sterile beaker.

II. *Eggs.*—Sterilize the shells of the eggs by immersing for ten minutes in 70 per cent. alcohol. Break the eggs into a sterile beaker, mix well and filter through sterile gauze. Add one part by volume of meat juice.

III. *Gentian Violet.*—Add sufficient 1 per cent. alcoholic gentian violet to make a dilution of 1 to 10,000.

Place in sterile test tubes and inspissate for three successive days. First day at 85° C. until all the medium is solidified. On the second and third days not more than one hour at 75° C.

Method of Isolating Tubercle Bacilli from Sputum

The use of fresh sputum is advisable. A mixture of the sputum and a 3-per-cent. sodium hydrate solution are left in the incubator for 20–30 minutes, then neutralized to sterile litmus paper with normal hydrochloric acid, centrifugalized and the sediment inoculated into the tubed media.

Method of Isolating Tubercle Bacilli from the Feces

The isolation of tubercle bacilli from the feces is not an easy problem. The concentration of the sodium hydrate is not as important as the length of exposure. The solid food particles are removed from the feces by dilution with water and filtration through gauze.

The filtrate is saturated with sodium chloride and at the end of half an hour all the bacteria will be found floating in a fine film. This film is collected and normal sodium hydrate added, shaken well and incubated at 37° C. for 3 hours. Then neutralized as is sputum, centrifugalized and sediment inoculated.

The method presented has proven very simple and accurate for the isolation of tubercle bacilli from the sputum. The partial success in isolating and cultivating tubercle bacilli from the feces may be due to the fact that many of the bacilli are possibly dead.

Comparative Analysis of Several Peptones: R. C. Colwell.

An investigation of the comparative merits of four brands of peptone is being made to determine the advisability of substituting an American brand for Witte's in Standard Methods of Bacteriological Analysis. The following table embodies the results of the chemical analysis.

	Total Nitrogen %	Ash %	Moisture %	Fermentation of Peptone Broth	Presence of Sugar	Acidity of 1% Peptone Broth
Witte's "Peptonum Siccum"	14.92	2.09	4.97	0	0	0.40
Digestive Ferments Co. "Peptone Powder Bacteriological"	15.83	4.18	2.96	0	0	0.24
Armour & Co. "Peptone Powdered containing 10% lactose"	13.67	5.76	4.09	+	[4]	0.60
Bausch & Lomb "Peptone from meat, dry"	12.82	4.32	6.06	+	[4]	0.56

The tests made upon the peptones to determine their relative reliability in the making of culture media has not yet covered long enough time to warrant any definite conclusions. However, it may be said that those peptones containing lactose seem to be inferior to those which are free from lactose.

A New Method of Precipitating Cellulose for Cellulose Agar: F. M. Scales.

The method of precipitating cellulose to be used for the preparation of cellulose agar is as follows: 100 c.c. of concentrated sulphuric acid are diluted with 60 c.c. of distilled water in a two-liter Erlenmeyer flask. The diluted acid should be cooled to about 60° to 65° C. Moisten with water five grams of filter paper, which are sufficient for one liter of cellulose agar, and add it to the acid, which should be vigorously agitated until the cellulose is dissolved. The flask is then filled as quicky as possible with cold tap water. The process of dissolving the paper and filling the flask requires about one minute. The precipitate may now be thrown on a filter and washed with distilled

[4] Lactose, about 10 per cent.

water until the filtrate no longer gives a test for sulphuric acid. As the volume of the suspension finally drains down to about 200 c.c. any deposit of cellulose on the filter may be removed with a camel's-hair brush. A hole is then punched in the bottom of the filter and the whole precipitate washed out and made up to 500 c.c., when it may be added to 500 c.c. of 1-per-cent. agar containing the nutrient salts. Cellulose-destroying bacteria were plated on a medium containing cellulose precipitated by this method and on one containing cellulose from Schweitzer solution. The destruction of the cellulose was about the same in both media.

New Technique for Studying Halophytic Organisms: K. F. KELLERMAN AND N. R. SMITH.

1. For staining flagella from salt media the bacteria are placed in a salt water suspension, killed by addition of 10 per cent. formalin, then placed in collodion dialyzing tubes and the soluble salts removed by dialysis. The bacteria are thrown down by centrifuging, and the residue spread on clean slides and stained by any method desired.

2. For isolating bacteria injured by heating to 42° C., use silica jelly. This can not be mixed with beef broth or peptone. When these nutrients are desired, pour sterile Petri plates of beef agar or peptone agar, allow them to harden for twenty-four hours, then for the isolating medium use synthetic salt solution and silicic acid solution, and pour this rapidly over the sterile beef or peptone agar plates and allow to remain perfectly quiet. The silica jelly forms a layer over the agar layer and the nutrients mix by diffusion.

3. Use collodion sacs to maintain constant supply of slightly soluble salts in clear solutions in bacterial culture flasks.

The Relative Merits of the Bubbling Method of Enumerating Air Bacteria: JOHN J. WENNER.

The writer is making a study of the modified Petri sand filter and the Rettger aeroscope bubbling filter for the purpose of determining their relative degree of efficiency, simplicity and practical value. The sand filter was, at first, set up as described in a previous paper by Weinzirl and Thomas ('12). As this apparatus was very cumbersome, it was soon modified by discarding one stopper entirely, holding the sand in the tube by means of a tight wire gauze, and attaching the aspirating tube directly to the main filtering tube. The great weakness of the sand filter is in the transference of the organisms caught in the sand, to the plate, so as to be easily and accurately counted. This was done in three ways: (1) The sand was distributed among several sterile plates and gelatin added. (2) The sand was transferred to a small sterile flask holding 10 c.c. of salt solution and an aliquot part plated. (3) The sand was transferred to a sterile test-tube holding 5 c.c. of salt solution, thoroughly washed, and the liquid plated with an equal amount of strong gelatin. This last method appears to be the most practical.

The Rettger aeroscope was used as originally described by Rettger ('10). A second plate, from washing the aeroscope and test-tube, should, in all cases, be poured.

The two filters were run simultaneously and consecutively under similar conditions. Air was taken in a dusty attic room and from a specially prepared box.

Both methods are equally simple and both filter with a high degree of accuracy. In plate pouring the aeroscope is simpler and contamination is not so easy. Besides the bubbling method is visible and audible, which may at times be very desirable. The writer's work has not been completed, but from his results thus far obtained the bubbling method gives him an excess in the number of colonies, over the sand filter. As technique is very delicate, a large number of tests have to be made for the results to be of any value.

One of the great drawbacks in the practical use of the air filter is the inconvenience of the aspirator. We need an aspirator that is easily transferred from place to place, one that is simple and yet will give a fair degree of accuracy, as well as a uniform and continuous rate of flow. For this purpose the writer has been experimenting by placing two movable tanks in a wooden box. The tanks are connected with a rubber tube while another tube from each tank extends to the outside. The filter is attached to the proper tube and the water passed from one tank to the other.

Suggestions for Partial Anaerobic Cultures: WARD GILTNER.

Anaerobiosis and aerobiosis are relative terms. The oxygen requirements and tolerance of microorganisms present a gradation from practically an absence of oxygen pressure to many times atmospheric pressure. The lowering of oxygen tension by biological means, Nowak's *B. subtilis* cultures, was introduced in connection with the growth of *Bact. abortus,* an organism requiring a slightly lower oxygen pressure than atmospheric. In this method the oxygen-consuming culture and *Bact. abortus* are usually grown in vitro separately, the two cultures being placed in a Novy or similar sealed jar. A simpler method is desirable. Re-

cently Geo. D. Horton[5] has proposed to grow both organisms on adjacent agar slants separated by a glass slide in the same test tube.

We suggest the following special tubes in which the culture surfaces may be kept separate while the air chamber is continuous or freely communicating between the sides. U tube with perforated corks and U capillary tube U and H tube. Probably the H tube will prove the most satisfactory. The communicating cross tube should be as short as possible so that the double tube may be held in the hand as conveniently as an ordinary test tube. Different media may be used on either side, either solid or liquid or a medium one side and some chemical on the other. The tubes should be plugged with rubber stoppers or sealed with paraffin or wax.

Dr. A. Parker Hitchens,
Secretary

(*To be continued*)

SOCIETIES AND ACADEMIES

THE BIOLOGICAL SOCIETY OF WASHINGTON

The 538th meeting of the society was held in the Assembly Hall of the Cosmos Club, Saturday, March 20, 1915, called to order by President Bartsch at 8 P.M., with 45 persons present.

Under heading Brief Notes, General Wilcox called attention to a Cedar of Lebanon near Jackson's statue in Lafayette Square.

The first paper of the regular program was by T. S. Palmer, "Notes on the Importation of Foreign Birds." The speaker discussed the subject with special reference to canaries, parrots and game birds. He stated that about 500 permits for importation of birds are issued annually by the Department of Agriculture, the number of birds imported a year amounts to about half a million; as high as 17,000 birds have arrived in a single day; the number of species imported is about 1,500; canaries constitute by far the largest number brought in. Methods of breeding birds, caring for them in transit, selecting and teaching singers and talkers were explained. Dangers of importing contagious diseases as the "quail disease" and methods of quarantining were pointed out. The effect of the European war on the importation of birds was commented upon. Dr. Palmer's paper was discussed by the chair, Dr. Stiles and Mr. Goldman.

The second paper was by Ned Dearborn, "Notes on the Breeding of Minks in Captivity." Among the habits of the mink attention was called to their profound diurnal sleep, cries emitted, polygamous nature, and cat-like character of food. The speaker stated that the period of gestation was found to be 42 days, number of young at birth 1 to 8; eyes of young remain closed for one month after birth; young may be weaned at 6 weeks; minks breed when a year old; and their fur is suitable for market at a year and a half, experiments showed that different types of diet had no effect on quality of fur. Speaker concluded that breeding of minks for commercial purposes was possible. Dr. Dearborn's paper was discussed by Messrs. Wetmore, A. B. Baker and Cooke.

The third and last paper was by M. W. Lyon, Jr., "*Endamœba gingivalis* and Pyorrhea." The speaker discussed the cause of pyorrhea or Rigg's disease, the *Endamœba gingivalis*, recently discovered by Dr. Allen J. Smith and others. He called attention to the pathologic lesions produced by the Endamœba and by the various bacteria associated with it; mentioned the amœbicidal action of emetin hydrochlorid administered systemically or locally; and reviewed some of the early references to the Endamœba before it was considered the cause of pyorrhea. The paper was illustrated by lantern slides of Gros's original drawing of the organism, and of several photomicrographs and drawings of living and stained Endamœbas, bacilli and spirochetes from a case of pyorrhea. Dr. Lyon's paper was discussed by Dr. Stiles and Mr. Goldman.

M. W. Lyon, Jr.,
Recording Secretary

Washington, D. C.

THE NEW ORLEANS ACADEMY OF SCIENCES

The annual meeting of the academy was held on Wednesday, March 10, in Stanley Thomas Hall, Tulane University. The following officers were elected for the coming year: President, Dr. Gustav Mann; First Vice-president, Dr. R. B. Bean; Second Vice-president, Dr. W. O. Scroggs; Treasurer, Mrs. E. J. Northrup; Librarian, Professor H. F. Rugan; Secretary, R. S. Cocks. The paper of the evening was read by Dr. C. W. Duval on "Modern Conceptions which Tend to Explain the Occurrence of Secondary Infection in Typhoid Fever and Tuberculosis." There was considerable discussion of the paper in which Drs. Mann, Lemann, Friedrichs participated. At the close of the meeting refreshments were served and the Academy adjourned.

R. S. Cocks,
Secretary

[5] *Jour. Inf. Dis.*, Vol. 15, No. 1, July, 1914.